Architectural, functional, and molecular responses to concentric and eccentric loading in human skeletal muscle

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Authors Contribution

Conceived and designed the experiments: MVN PJA NDR MF. Performed the experiments: MVF NDR WKM. Analysed the data: MVF AS RMBV. Contributed reagents/materials/analysis tool: MVN PJA MF JW. Wrote the paper: MVF MVN PJA NDR MF.

Running head: Muscular responses to concentric vs. eccentric training in young men

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ABSTRACT

Aim: We investigated architectural, functional, and molecular responses of human skeletal muscle to concentric (CON) or eccentric (ECC) resistance training (RT). ***Methods:*** Twelve young males performed 10 weeks of concentric (CON) or eccentric (ECC) resistance training (RT) (n = 6 CON, 6 ECC). An additional 14 males were recruited to evaluate acute muscle fascicle behaviour and molecular signalling in biopsies collected from vastus lateralis (VL) after 30 min of single bouts of CON or ECC exercise. VL volume was measured by magnetic resonance imaging. Muscle architecture (fascicle length, Lf; pennation angle, PA) was evaluated by ultrasonography. Muscle remodelling signals to CON or ECC loading (MAPK/AKT-mammalian target of rapamycin (mTOR) signalling) and inflammatory pathway (TNFα/Murf-1-MAFbx) were evaluated by immunoblotting.

Results: Despite the ~1.2 fold greater load of the ECC group, similar increases in muscle volume (+8% CON and +6% ECC) and in maximal voluntary isometric contraction (+9 % CON and +11 % ECC) were found after RT. However, increases in Lf were greater after ECC than CON (+12 vs. +5%) while increases in PA were greater in CON than ECC (+30 vs. +5%). Distinct architectural adaptations were associated with preferential growth in the distal regions of VL for ECC (+ECC +8% vs. +CON +2) and mid-belly for CON (ECC +7 vs. CON +11%). While MAPK activation (p38MAPK, ERK1/2, p90RSK) was specific to ECC, neither mode affected AKT-mTOR or inflammatory signalling 30 min after exercise. ***Conclusion***: Muscle growth with CON and ECC RT occurs with different morphological adaptations reflecting distinct fibre fascicle behaviour and molecular responses.

Introduction

Skeletal muscles can contract by shortening (concentric) or lengthening (eccentric) (Joyce et al. 1969; Joyce & Rack 1969). "Conventional" resistance exercise training, using commercial exercise machines is the most common form of resistance-exercise, consisting of lifting and lowering a constant external load. Thus, conventional resistance exercise training combines CON (lifting-phase) and ECC (lowering-phase) actions. According to the force-velocity (F-V) relationship, each value of force and velocity on a given curve should belong to the same level of neural activation (Bigland & Lippold, 1954; Camilleri & Hull, 2005; Chow & Darling, 1999). Yet, this requirement is not met by conventional RT as the same external load is displaced during both lifting and lowering phases. Thus, motor units must be de-recruited in the ECC part to enable the load to be lowered (Reeves et al. 2009); as such the load used for conventional training is limited by the CON muscle action. Therefore, to ensure that the ECC component of resistance training is not under-loaded, it would be necessary that both shortening and lengthening phases follow the physiological force-velocity curve i.e., the absolute load should be greater for the ECC than the CON contraction (Katz 1939), theoretically involving the same level of neural activation between contraction modes. Nonetheless, to our knowledge comparisons of pure CON to ECC exercise with such matching to equalize the relative loading stimulus, meeting a fundamental premise of the F-V relation, have not yet been made.

A recent investigation (Reeves et al. 2009) provided evidence that distinct loading patterns also lead to distinct architectural adaptations to exercise training, as suggested by Hortobágyi *et al.* (1996). In this previous study (Reeves et al., 2009), the architectural responses to muscle loading in older-aged individuals undergoing conventional vs. ECC

only training regimes were compared. After 14-wk of training the authors noted a greater increase in muscle fibre (fascicle; Lf) length in the ECC only group compared to the conventional RT group. Conversely increases in pennation angle (PA) were only evident following conventional RT, but not after ECC only exercise. Furthermore, since conventional RT involves mixtures of both CON and ECC contractions, the architectural responses to "pure" ECC or CON contractions performed on standard isotonic machines with such matching for relative loading stimulus are unknown.

Distinct architectural adaptations to ECC vs. CON contractions also raise the question as to what could be the molecular basis of this phenomenon. Since both human and pre-clinical work has provided evidence of distinct molecular responses to e.g. CON vs. ECC contractions, it is likely that similar mechanisms underlie the different architectural adaptations. This hypothesis seems supported by the recent observation that ECC vs. CON growth of cardiomyocytes is regulated via ERK1/2 MAPK signalling (Kehat et al. 2011), demonstrating that acute signalling differences in response ECC vs. CON exercise could underlie the ensuing distinct architectural adaptations. In another investigation, using isolated rat muscle Wretman et al. (Wretman et al. 2001) reported greater increases in phosphorylation of ERK 1/2 and p38 MAPKs induced by ECC vs. CON contractions. In addition Martineau et al. (Martineau & Gardiner 2001), observed that activation of MAPKs activation was quantitatively related to muscular tension with ECC contraction providing the greater stimulus. Finally, microarray analyses in young men (Kostek et al. 2007) demonstrated distinct responses to *acute* CON vs. ECC contractions, suggesting that contraction-specific muscle remodelling results both from distinct signalling and genomic responses to CON vs. ECC exercise. Nonetheless, the relationships between MAPK (or other) signals and that of the distinct architectural

basis of skeletal muscle hypertrophy in response to CON vs. ECC exercise, remains unknown.

Therefore, the aim of the present study was to compare the effects of pure CON vs. ECC exercise training in terms of architecture, morphology and functional outcomes, and relate this to muscle cell signalling responses potentially ascribing the distinct structural and functional adaptations to CON vs. ECC training. The hypothesis put forward was that different mechanical stimulus (shortening vs. lengthening), chronically applied and matched to balance the relative loading inducement, would result in distinct adaptations in muscle morphology, function and architecture: possible underlying mechanical and biochemical mechanisms may be involved in these distinct remodelling processes.

METHODS

We recruited 12 young men (25±3 y, height = 182±8.5 cm, mass = 71.9±8.5 Kg; means ± SD) not partaking in resistance exercise training to undergo a 10-week resistance exercise-training program. Based on their maximum isometric knee extension torque, they were divided (matched for baseline strength) into two training groups: EG (ECC, n=6, 25±3 y) or CG (CON, n=6, 25±3 y). Resistance exercise training was carried out with a leg-press machine (Technogym, Gambettola Italy) modified to enable performance of either an ECC only (EG) or CON only (CG) contractions. This was achieved using an electric engine attached to the back of the leg-press (Fig 1): in the EG, the chair was pulled back with a cable that connected the electric winch to the weight stack via a steel cable, ensuring that subjects did not exert any force with their quadriceps to perform what would otherwise have been the concentric component of the exercise. When the chair was released, it enabled the subject to lower the training load under control

through an ECC contraction of the quadriceps. Conversely the CG performed a CON-only movement consisting of lifting the load. In this case the engine operated only during lowering of the load, ensuring that subjects did not exert any force with their quadriceps to perform what would have otherwise been the eccentric component of the exercise. The timing of the contraction was slightly different for the two groups: the CG were asked to complete the contraction in ~2 s, whereas this time period was ~3 s for the EG. This time difference (~2 s CG *vs* ~3 s EG) was necessary to ensure that the load was indeed lowered under control in the EG. The training period for the first study was performed, after a familiarisation session, three times per week for 10-wk and people trained both legs but unilaterally. Both training and acute exercise bouts on the leg press machine involved the main extensor muscles of the lower limbs. The training load used was of 80% of the concentric (CG) or 80% of the eccentric (EG) 1RM, with 4 series of a minimum of 8 to a maximum of 10 repetitions with one-minute rest in between the sets. The 1RM was assessed unilaterally after a warm-up program performed on the leg press machine using a very light weight that allowed the subject to easily perform 8 to 10 repetitions (concentrically or eccentrically: lifting or lowering phase, respectively). Then, the protocol followed for both contraction phases was the one suggested by Baechle & Earle (1994). This study was approved by the ethical committee of the health care science faculty of the Manchester Metropolitan University and conformed to the requirements of the Declaration of Helsinki. Volunteers were informed of the purpose of the study, the experimental design and procedures involved and all the potential risks involved before giving their written consent

Measurement of electromyographic (EMG) activity

VL integrated EMG was measured as representative of the knee extensors to provide an indication of neural drive to this muscle group during the tests performed on the Cybex dynamometer. Two surface electrodes (10 mm diameter) were placed next to each other on the lower third of the VL muscle with a 20 mm centre-to-centre electrode distance. These two electrodes were arranged in a "bi-polar" configuration with a third electrode, the "ground", placed on a bone area (the patella bone in this case). The skin was shaved and conditioned using a special skin preparation gel (Nuprep™) to reduce skin impedance (using an electrode impedance tester - Oxford medical ltd, Medilog, UK) below 5,000 Ohms. In order to reproduce the same electrode positioning in the successive recording sessions, measurements were taken and anatomical spots (bone processes, tendon and muscle insertions) were used to know exactly the right portion of VL for the surface electrodes to be placed. Acquisition of the surface EMG signal was obtained through the Biopac A/D acquisition system at a sampling frequency of 2000 Hz –and filtered through a bandwidth of 10-500 Hz. The root mean square (RMS) was calculated from the raw EMG over a 200 ms time frame where the peak of torque was expressed during the isometric MVC trials. During the 1RM assessment, EMG was monitored in order to support our assumption that CON and ECC 1RMs would have resulted in similar neural drive: the RMS was calculated over a 200 ms time frame during the mid-portion of the contraction phase.

Magnetic Resonance images (MRI)

Axial plane scans of the thigh were taken before (1 week) and post-training (4-5 days) using a 0.25 Tesla magnetic resonance imaging (MRI) scanner (Esaote G-scan, Italy). A T1-weighted Spin Echo protocol was used (repetition time 900 ms, echo time, 26 ms, number of excitation 2, Field of View 200x200 mm, slice thickness 10 mm, gap between

slices, 1.0 mm). Participants were asked to lie supine on the MRI bed and to insert their leg into a circular coil. Due to the scanning area of the coil, the thigh was imaged in 3-4 separate sections. Markers were placed on the thigh from the patella to the hip to denote different sections and avoid overlap. Axial plane scans along the entire length of the VL were collected; on average, the number of axial scans obtained in each subject was the same for the baseline and post-training periods (~34). From these scans the contours of the VL muscle of each MRI scan were digitized using the Osirix image analysis software and, subsequently, VL Muscle Volume was calculated as follows:

$$\text{Volume}_{VL}\ (cm^3) = \Sigma_{ACSA} \cdot (\text{slice thickness} + \text{gap between slices}).$$

Regional VL hypertrophy was calculated after training by obtaining the baseline and post-exercise average values of the first 5 axial scans where the VL muscle was visible starting from the hip/knee joint (proximal and distal portions respectively) and the 5 scans around the peak of ACSA (muscle mid portion): from these mean values, the percentage increase in ACSA was calculated for the 3 different regions of the VL muscle.

Muscle (VL) Architecture

Before (1 week) and after training (4-5 days), VL muscle architecture i.e., Lf and PA were measured (by the same investigator) from images obtained *in vivo* at rest using B-mode ultrasonography (Mylab 70, Esaote Biomedica, Italy), with a 100 mm, 10-15 MHz, linear-array probe. Resting ultrasound images were taken at a specific joint angle (150°), corresponding almost to full knee extension (180°), while the participant was seated on the Cybex Norm dynamometer chair; the transducer was aligned in the fascicle plane in

order to be able to visualize an optimal portion of fascicles on the ultrasound screen. The muscle architectural parameters were quantified from the ultrasound scans using the image analysis software, ImageJ 1.42q (National Institutes of Health, USA). The visible portion of the fascicle length was directly measured using this software. In some instances, a small portion of the fascicle extended off the ultrasound window and it was necessary to estimate this non-visible portion using a linear extrapolation of fibres and aponeuroses (Erskine et al. 2009). Pennation angle was measured as the intersection between fascicles and the deep tendon aponeurosis (Fig 2). The reliability of these ultrasound techniques has been published (Intra Class Correlation value = 0.99)(Reeves et al. 2004) ; images were collected and digitally analysed by the same operator.

Muscle function

Participants were familiarized with all the devices and procedures involved in the study before the actual test sessions: the exercise-training participants were asked to perform contractions in a seated position on the reclining chair of the Cybex Norm dynamometer (hip angle = 85°, hip angle at supine position = 0°). The lower leg was strapped to a pad situated at the end of the Cybex lever arm and the knee joint center of rotation was aligned with the dynamometer fulcrum. The torque produced on the Cybex dynamometer was sampled into an analogue to digital acquisition system (Biopac System, Inc. California) at a frequency of 200 Hz and displayed on the screen of an Apple computer (Mac. G4). Maximum isometric torque of the knee extensor muscle group was evaluated by participants performing an isometric maximum voluntary contraction (MVC) at every 10º (0.175 rad) from 90° to 150° (from 1.57 to 2.62 rad) of knee joint angle (180° = full extension). Two MVCs were recorded at each joint angle with 2 min

separating each contraction and the highest torque produced was used to assess MVC changes from pre to post training.

Acute behavioural and molecular responses to CON and ECC contraction

An additional untrained 14 men (25±4 y, height = 184±7 cm, mass = 74±4 Kg) were recruited and divided into two groups (CG acute, n=7, 26±4 y and EG acute, n=7, 25±4 y) to perform a single bout of ECC or CON exercise, adopting the same design of the training study (same load-repetitions-sets combination). Vastus lateralis (VL) muscle biopsies were collected in these additional volunteers before and 30 min after exercise for signalling purpose: this time was specifically chosen as MAPK activation appears to be transient (Nader & Esser 2001). Ultrasound scans were also acquired during a single CON or ECC contraction performed on the leg-press device. Measures of fascicle length and pennation angle were recorded from screen captures during contractions and analysed in an identical fashion to in the training study.

Immunoblotting

Post exercise biopsies were processed in a similar fashion to as previously described (Atherton et al, 2010). Briefly, ~20 mg of muscle was snipped in ice-cold buffer (50 mM Tris-HCl (pH 7.4), 50 mM NaF, 10 mM β-Glycerophosphate disodium salt, 1 mM EDTA, 1 mM EGTA, 1 mM activated Na_3VO_4 (all Sigma-Aldrich, Poole, UK)) and a complete protease inhibitor cocktail tablet (Roche, West Sussex, UK) at 10 $\mu l.\mu g^{-1}$ of tissue. Homogenates were rotated for 10 min and the supernatant collected by centrifugation at 13,000 × *g* for 5 min at 4°C. The supernatant (sarcoplasmic fraction) was used for immunoblot analysis: protein concentrations were determined using a NanoDrop ND1000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE-US) and adjusted to $1\mu g.\mu l^{-1}$ in 3× laemmli. Each sample was loaded onto pre-cast 12% Bis-Tris

Criterion XT gels (BioRad, Hemel Hempstead, UK) at 15 μg per lane and separated electrophoretically at 200 V for 1 h. Proteins were then wet-transferred at 100 V for 1 h onto polyvinylidene difluoride (PVDF) membranes (0.22 μm), blocked for 1 hour in 2.5% skimmed milk in 1× Tris-buffered saline/ Tween-20 (TBS-T), and then incubated in 1° antibodies (1:2000 dilution in 2.5% BSA in TBS-T) rocking overnight at 4°C. For phosphorylation of MAPK p38 (Ser189/207), p90RSK (Thr359/Ser363), ERK1/2 (Thr202/Tyr204), p70S6K (Thr389), Akt (Ser473), p65 (Ser536) and pan-actin antibodies were obtained from Cell Signaling Technology, Inc. (MA, US), 4E-BP1 (Ser65/Thr70) from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA-US) and MAFbx, Murf-1 (C-terminal region) from ECM Bioscience (KY, US). For total amount of TNFα, p65 and IkBα antibodies were obtained from Cell Signaling Technology, Inc. (MA, US) The next day, membranes were washed 3×5 min in TBS-T, incubated in HRP-conjugated 2° antibody (New England Biolabs, Hertfordshire, UK; 1:2000 in 2.5% BSA in TBS-T) at room temperature for 1 h, before 3×5 min washes in TBS-T. Membranes were exposed to chemiluminescent HRP Substrate (Millipore Corporation, Billerica, MA-US) for 5 min and bands quantified by Chemidoc XRS (BioRad, Hertfordshire, UK). Software measures were taken to prevent pixel saturation; loading anomalies were corrected to Pan-Actin.

Statistical analysis

Differences for group (CG *vs.* EG, the training groups and CG1 *vs.* EG1, the acute study groups) and time (baseline *vs.* post-training / baseline *vs.* post-exercise) were analyzed using a two-way factorial analysis of variance test using GraphPad PRISM software (version 5.0d; GraphPad software Inc. San Diego, CA). Significant interactions between groups and time were located by Bonferroni post-hoc test. The delta (Δ) training values

(percentage increases) were statistically tested between groups using an independent t-test, that was also used to compare baseline differences between CG and EG for physiological parameters. A power calculation was performed: our current sample size has a beta level of 0.8 (i.e., power of 80%) for the training study (12 participants) using the parameter of pennation angle and a beta level of 0.9 (i.e., power of 90%) for the acute study (14 participants) using the parameter of p38 MAPK.

RESULTS

EMG of CON-ECC 1-RM, maximum lifting or lowering ability (1-RM) and training load

As mentioned, to test our assumption that both CON and ECC contractions belonged to the same Force-Velocity curve, EMG activity was measured during performance of a single concentric and eccentric 1-RM in each subject to evaluate if the two phases correspond to a similar level of neural activation. The means of baseline EMG values for CON and ECC group 1-RM are presented in Table 1. These values represent the mean of the individual rectified EMG activity measured during the entire CON or ECC 1RM and were collected just prior to the training period. In support of our assumption, no significant difference existed in neural activation during the performance of the CON or ECC only exercise. As expected, regarding the maximum lifting or lowering ability data, the baseline and post-training 1-RM was higher in the ECC than the CON group (Table 1), resulting in a higher ECC training load and consequently higher training volume, (132592 vs. 105120 Kg, $P < 0.01$, calculated as number of sets X number of repetitions X training load for ECC compared to CON exercise in the 10-wk period). The pre–to-post training increase in 1-RM was statistically significant in both groups, but with no significant difference in the percentage increase between the ECC and CON group.

Muscle morphology and architecture and maximum voluntary contraction

After training, both groups showed an increase in VL muscle volume but the change was similar between the EG (6 ± 0.4%, mean ± SEM, $P < 0.0001$) and CG (8 ± 0.5%, $P < 0.0001$). However, Lf increased significantly more ($P < 0.01$) in the EG (12 ± 2%, $P < 0.0001$) compared to the CG (5 ± 1%, $P < 0.01$); conversely PA increased significantly less ($P < 0.01$) in the EG (5 ± 1%, $P > 0.05$) than the CG (30 ± 0.5%, $P < 0.0001$) group. Maximum voluntary contraction (MVC) peak amplitude changed in both groups similarly (significant pre-to-post difference, $P < 0.05$) (EG = 11 ± 8%, $P < 0.05$, CG = 9 ± 6% increase, $P < 0.05$; Figure 3).

Regional hypertrophy of VL muscle in response CON or ECC training

Differences in localized hypertrophy were observed in response to 10-wk of either CON or ECC resistance exercise (Figure 4). While both loading modalities induced similar effects on ACSA % increase/decrease in the proximal area (EG = -1 ± 1%, mean ± SEM, and CG = -0.5 ± 1%) a significant difference was found in both mid portion (EG = 7 ± 1%, and CG = 11 ± 1%, $P < 0.01$) and distal part of vastus lateralis (EG = +8 ± 2% versus CG = +2 ± 1.5%, $P < 0.05$) between the two types of training.

Architectural behaviour of VL muscle during performance of CON vs. ECC contractions

Following discovery of such distinct architectural adaptations, we recruited a second cohort to interrogate possible mechanical reasons for these findings, with the aim of determining fascicle behaviour during CON and ECC contractions. Differential behaviour was observed in Lf and PA during CON and ECC resistance exercise performed with leg-press (Figure 5). During ECC, fibres lengthened during performance of ECC exercise (Lf =

+19 ± 2%, mean ± SEM, from start to end of contraction, $P < 0.0001$) whereas during CON there was a substantial fascicle shortening in Lf (Lf = -19 ± 2%, $P < 0.0001$). Similarly, while PA remained similar from the start to the end of ECC (PA = -3 ± 1%, $P > 0.05$), it showed a substantial increase during CON (PA = +28 ± 1%, $P < 0.0001$).

Acute MAPK, AKT-mTOR and Inflammatory/breakdown signaling responses to a single acute bout of CON vs. ECC exercise

By taking biopsies 30 min following this single bout of CON or ECC we were also able to interrogate intramuscular signalling purported to be involved in exercise adaptations. Significant increases in phosphorylation of mitogen activated protein kinases (MAPKs) i.e., p-38MAPK, ERK1/2 and p90RSK (Figure 6) were found 30 min after ECC resistance exercise (p38MAPK = 20 ± 4-fold, ERK1/2 = 2 ± 0.3-fold, p90RSK = 3 ± 1-fold) but not after CON resistance exercise. In contrast, there was no modulation in the phosphorylation of Akt (Ser473) and mammalian target of rapamycin (mTOR) substrate p70S6K 30 min after CON or ECC exercise, although a significant suppression ($P < 0.05$) in activation of 4-EBP1 was found only after CON exercise (Figure 7). Non-significant changes in the activation of the TNFα/Murf-1-MAFbx pathway (TNFα, p-p65, p65, IkBα, p-MurF-1, p-MAFbx) were found 30 min after CON or ECC exercise.

DISCUSSION

In the present study we compared, for the first time, the structural remodelling of human skeletal muscle in response to pure CON and ECC loading while attempting to link these with the molecular signalling pathways implicated in muscle remodelling (MAPKs, mTOR etc). Furthermore, whilst previous investigations focused on

morphological and architectural responses to CON and ECC resistance training matched for work-load (Blazevich et al. 2007; Higbie et al. 1996; Moore et al. 2012), no study has yet, to the best of our knowledge, matched the CON and ECC phases for the same relative load while monitoring neural drive in order to meet one of the fundamental requirements of the F-V relationship (Bigland & Lippold 1954; Camilleri & Hull, 2005; Chow & Darling, 1999). Hence, our training loads were matched to the same percentage of the CON and ECC repetition maximum (i.e., CON 1RM and ECC 1RM) and EMG values revealed similar levels of neural activation for both CON and ECC 1-RM (Table 1). The ECC group/CON group training load ratio remained between the 1.21 to 1.29 range (Table 1), this confirms previous observations of the greater forces associated with ECC than CON *in vivo* (Aagaard et al. 2000; Westing et al. 1988). These findings support our contention that both shortening and lengthening phases of our resistance exercise paradigms belonged to the same F-V curve. Although 1RM assessment is a sort of an "unrefined" method, the fact remains that 1RM is still recognised as 'gold standard' in training studies. Furthermore, the best available technique to assess neural drive in vivo is still EMG. In this investigation, integrated EMG (i.e. result of recruitment and rate coding) was similar in the two different conditions. The authors would like to emphasise that in the present study the load was not matched for neural activation but rather, EMG was recorded in relatively equal external loads and similar EMG values were found.

Muscle hypertrophy *per se*, was an expected consequence of our resistance training protocols. However, muscle volume showed similar changes after both training modes (ECC = +6 and CON = + 8%, non significant difference between groups). Although both concentric and eccentric exercise programs have shown to induce gains in muscle mass there seems to be insufficient evidence of the superiority of either these two types of

337 contraction (Wernbom et al. 2007). Nevertheless, the similar changes in muscle volume
338 in the present study were considered unexpected, as not only ECC training load was
339 higher, but also because ECC training has been suggested to produce greater
340 hypertrophy and strength than CON training (increase in muscle fibre size, Hortobágyi
341 et al. 1996) and associated trends towards whole muscle greater CSA (Roig et al. 2009).
342 If the predominant promoter of muscle hypertrophy were the mechanical stimulus, one
343 would expect to find a greater hypertrophy in the ECC group due to the higher training
344 load (i.e. higher mechanical stimulus). However, this was not the case, indicating that the
345 intensity of mechanical stimulus may not be the sole determinant for muscle
346 hypertrophy; rather, this might be governed by the type of contraction performed
347 (ECC/CON) and also that other factors blunting muscle hypertrophy may be at play in
348 ECC contractions.
349
350 Interesting differences in muscle architecture using ultrasound were also found as result
351 of the different training regimens. Although Lf increased in both groups, the ECC group
352 showed a significantly greater gain in Lf compared to the CON one, while training
353 produced an increase in PA after both types of training but the increase in pennation
354 angle in the ECC group was much lower than in the CON one (Fig. 3). These findings
355 suggest that addition of serial sarcomeres occurs in response to muscle lengthening
356 scenarios (e.g. Holly et al. 1980; Reeves et al. 2009; Seynnes et al. 2007), and herein
357 mainly, as a result of the ECC component. Instead, increases in PA occur to bundle more
358 contractile units along the tendon aponeurosis (Gans & Bock 1965; Kawakami et al.
359 1993) primarily reflecting muscle shortening, principally as a result of the CON
360 component. Finally, while these findings of distinct architectural responses are allied
361 with those reported by Reeves et al. (Reeves et al. 2009) in older humans after ECC vs.

362 conventional training, our current evidence for distinct architectural adaptations to *pure*
363 CON vs. ECC in younger individuals is the first report of its kind and reveals that
364 adaptations following conventional RT are dominated by the concentric component (at
365 least in older men), perhaps reflecting the greater loading stimulus of the CON phase
366 compared to the ECC one, when applied using standard gym equipment.
367
368 Intriguingly, while both groups showed a similar overall increase in VL muscle volume,
369 the regional morphological patterns of muscle hypertrophy induced by the two loading
370 modes differed substantially. For instance, while ECC exercise promoted greater muscle
371 hypertrophy (as measured by changes in ACSA by MRI) in the distal portion compared
372 to CON, increases in the mid VL muscle were greater for CON than ECC (Fig. 4). We
373 contend that evidence of these differences in the regional distribution of hypertrophy
374 along the muscle belly, reflect a differential addition of sarcomeres in series and in
375 parallel. Pennation of muscle fibres allows greater packing of sarcomeres in parallel
376 along the tendon aponeurosis (Gans & Bock 1965). Hence, the finding that CON training
377 promoted a large increase in pennation (30%), with little increase in fascicle length
378 (5%), strongly suggests that CON training leads to hypertrophy mainly through addition
379 of sarcomeres in-parallel. As indicated by the increase in ACSA, this phenomenon seems
380 to mainly occur in the central region of the VL, which, because of the bell-shaped
381 distribution of muscle ACSA, comprises a large portion (~ 60%) of the whole VL volume.
382 Instead, when training involved muscle stretch, i.e. with ECC training, hypertrophy
383 occurred mainly through an elongation of fascicles (12%) and with little increase in
384 pennation angle (5%), suggesting preferential addition of sarcomeres in-series. The
385 increase in ACSA over a larger portion (about 2/3) of the muscle belly (central and distal
386 regions) associated with preferential increase in fascicle length suggests that the

addition of new sarcomeres in series occurred over a large portion of the muscle belly. It remains to be established where along muscle fibres sarcomere were added but it is probable that this occurred at the periphery since early (Williams & Goldspink 1971) as well as recent (Allouh et al. 2008) observations showed (directly or indirectly), preferential addition of sarcomeres in series at the periphery of muscle fibres in response to stretch overload, and in response to developmental growth, as satellite cell frequency and concentration seems particularly high at the ends of muscle fibres (Allouh et al. 2008). Although this accordance between architectural and morphological adaptations to training seems reasonable, a limitation of the present study is that ultrasound scans were taken just from the middle of the muscle belly with the assumption that changes in architecture observed in this region would be representative of changes along the whole muscle. This may not be the case, as pennation angle might have increased more closer to the myotendineous junction after ECC exercise (i.e. causing the greater VL distal hypertrophy). However, although in principle it could be argued that limiting the ultrasound scans to a single muscle site may not also be representative of other changes occurring in other muscles, it must be acknowledge that Vastus Lateralis presents a more uniform architecture throughout its length compared to other heads of the quadriceps (i.e. Vastus intermedius (VI) presents inhomogeneous architecture, Blazevich et al. 2006). Moreover, a very recent publication investigating the changes in muscle architecture between different sites of the four heads of the quadriceps, observed how the adaptations in muscle CSA, thickness and PA were quite consistent between the Vasti and only significantly different if compared to Rectus Femoris (RF) changes size and architecture (Ema et al. 2013). These results could be explained by the fact that RF is a bi-articular muscle, differing from the vasti anatomically and biomechanically. The region of VL investigated in the present study

(VL mid length) coincides with the site in which the largest CSA value was observed. Furthermore, our aim was to show different responses brought by the two different loading paradigms: the choice of the muscle site is supported by the study by Ema et al. (2013) in which VL is the muscle that showed less inhomogeneous changes between CSA and architecture throughout the muscle hence we have reason to believe that this site could still be the best representative of the whole quadriceps.

Blazevich and colleagues (2007) similarly reported an increase of Lf in response to ECC exercise in the first 5 weeks of training but it could be argued whether the architectural adaptations of the present study do continue overtime, as it appears that in Blazevich's study these adaptations did not occur beyond the 5 weeks period. Thus, whilst Blazevich and colleagues confirmed the early architectural adaptations phenomenon previously reported by Seynnes et al. (2007), our present work suggests that these changes are still detectable after 10 weeks of RET. Further investigation is needed in order to assess if these architectural responses will be observed of different magnitude (i.e. similar or milder) after 5 weeks of RET.

Despite the distinct global (i.e., whole-muscle volume) hypertrophy responses between CON and ECC training groups, functional (strength) adaptations revealed similar increases in isometric MVC for both groups (CON 9%; ECC 11%). Although this similarity in the strength increase seems paralleled by the changes in muscle volume, it does raise the question of why, despite the greater training load (1.2 fold) of the ECC group, VL hypertrophy was similar. Possible causes of this finding and of the different architectural adaptations to ECC and CON training may be linked to muscle damage

caused by ECC contractions and to distinct signalling pathways involved in ECC and CON.

Following such intriguing findings in the adaptive features of VL muscle after 10-wk of CON vs. ECC, we chose to investigate the acute changes of muscle architecture in response to single CON or ECC exercise bouts by recruiting a second subject group. As expected, based upon the behaviour of the muscle-tendon unit, during CON contractions fascicles shortened (by -19%) and lengthened (+19%) during ECC contractions. We speculate that the contraction-specific VL fascicle length change (i.e., marked fascicle shortening during CON and marked fascicle lengthening during ECC) is a primary cause of the differential architectural adaptations and that such adaptations start from the first training session after CON and ECC bouts, as suggested by Seynnes *et al.* (Seynnes et al. 2007) who showed that such differences in muscle architecture can be detected at very early stages of training.

Although distinct cell signalling responses to CON and ECC in humans have yet to be established, in the present study we observed increases in phosphorylated MAPK e.g. p-38 MAPK, ERK 1/2 and p90RSK in the ECC but not CON. Similarly, reputed differences in the signalling response of muscle cells have been observed in animal models (Martineau & Gardiner 2001; Wretman et al. 2001). Furthermore, ERK1/2 expression was previously shown to regulate CON vs. ECC growth pathways in cardiomyocytes, suggesting MAPKs are involved in regulating architectural remodelling processes in muscle tissue (Kehat et al. 2011). In this latter study, cardiomyocytes isolated from mice lacking ERK showed an increase in length of the cardiomyocytes (ECC growth) whereas cardiac cells isolated from mice over-expressing MEK1 (a MAPK-Kinase, ERK1/2 up-

460 regulator) showed a preferential growth in myocyte thickness (CON growth).
461 Recruitment of this second study group allowed us to interrogate the effects of an acute
462 bout of CON or ECC upon intramuscular signalling proteins associated with adaptation
463 to exercise, with particular focus on MAPK's. In doing this, we observed a specific MAPK
464 activation only in response to ECC (which lead to a preferential increase in Lf), although,
465 a similar and simple relationship between ERK1/2 and determination of architectural
466 adaptation in human skeletal muscle could not be confirmed, unless it is the reverse of
467 the mechanisms occurring in cardiac muscle. Further work is needed to define this.

468 Another clue as to why these architectural differences may exist is that ECC leads to a
469 greater degree of damage than CON (Byrne et al. 2004), with greater myofibrillar
470 disruption occurring with ECC (Schoenfeld 2012). However, we found no significant
471 increases in TNFα/Murf-1-MAFbx pathway (p-TNFα, p-p65, p65, IkB, p-MurF-1, p-
472 MAFbx) 30 min after exercise. This supports the notion that activation of MAPK in
473 response to ECC occurred independently of muscle damage/inflammation, acting
474 through MAPK (Kramer & Goodyear 2007; Murton et al. 2008) and more likely through
475 mechano-transduction mechanisms (although we cannot exclude the likely onset of
476 muscle damage/inflammation phenomena after our single biopsy time point).
477 Furthermore, despite the fact that we observed no differences in whole-muscle
478 hypertrophy between ECC and CON (despite the marked architectural adaptive
479 differences), we measured activation of growth signalling (mTOR substrates). Apart
480 from suppression of 4EBP1 30 min post CON only (Atherton et al. 2005), no other
481 signals were modulated by 30 min post CON or ECC. Clearly, the energy stress after
482 exercise, which governs the latency in muscle protein synthesis responses (Cuthbertson
483 et al. 2006), may have prevented us from evaluating MAPK and AKT-mTOR cross-talk.

However, no further biopsies were taken, which represents a study limitation. It must be acknowledge that performing acute and chronic studies in different groups precluded interrogation of correlative links e.g. between MAPK phosphorylation and architectural adaptations. Nonetheless, the acute exercise data revealing substantial contraction-dependent divergence in mechanical and molecular responses and the chronic data revealing divergent architectural/ morphological adaptations are highly robust. It could also be argued that one limitation of this study is the different time under tension curve found in the two types of contractions: as stated, the greater time under tension in the eccentric mode was specifically chosen to enable to perform the lengthening/lowering of the load phase in safety. Nevertheless, as previous studies have shown (Burd et al. 2012) the greater time under tension curve is associated to increased anabolic response. If this was the case in this study, we should have observed differences in hypertrophic response (i.e. ECC Vol > CON Vol), which did not occur. Burd and colleagues investigated the anabolic responses to different time under tension comparing 1 second contractions vs. 6 seconds ones (6-fold greater) whereas the present investigation used 2 sec vs. 3 sec (0.5-fold difference): it is likely that this relatively smaller difference in time under tension was not sufficient to trigger different hypertrophic adaptations, as the morphological data are much more reflecting the findings presented by Adams et al. (2004) that showed same increase in muscle size to ECC, CON and isometric training of the same duration. Moreover, although the greater time under tension could reflect/suggest a likely increased physiological blood flow restriction occurring during the ECC phase, which should therefore result in a higher stimulation of hypertrophic signalling and/or greater muscle volume (Meyer et al. 2006), in this study no differences have been found in terms of anabolic signalling between the two training regimes.

Hence we may conclude that this difference in time under tension was not sufficient to modulate a differential anabolic response.

Conclusions

This study has shown that CON and ECC training paradigms lead to divergent structural adaptations, supported by different myogenic responses. ECC training leads to a marked increase in fascicle length (~1.5 fold) with no significant change in pennation angle while CON training induces a 3-fold increase in pennation angle, with little (<1-fold) change in fascicle length. These results suggest that ECC training seems to promote the addition of sarcomere in series whereas CON training favours the addition of sarcomere in parallel. This differential pattern of sarcomere addition induced by the two types of training, as inferred by the increase in fascicle length and pennation angle, seems also reflected by the distribution of muscle hypertrophy along the VL muscle belly, predominant in the mid to distal regions for ECC training and predominant in the mid-belly region for CON training. The different muscle remodelling induced by CON and ECC training may be associated with distinct MAPK responses to the two contraction modes. The similar hypertrophy with ECC and CON RT may be explained by the greater myofibrillar disruption caused by ECC loading, followed by possible activation of inflammatory pathways likely antagonizing muscle hypertrophy.

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FIGURES

Fig 1. Subject on the Technogym leg press modified ad hoc with the special electric engine visible on the right corner indicated by the arrow (Fig 1 A). The electric winch attached to the chair via steel cable; Fig 1B shows how the winch was connected to the chair (the red arrow indicates a counterweight that prevented the cable from becoming too slack and getting damaged); Fig 1C presents the site where the engine was placed

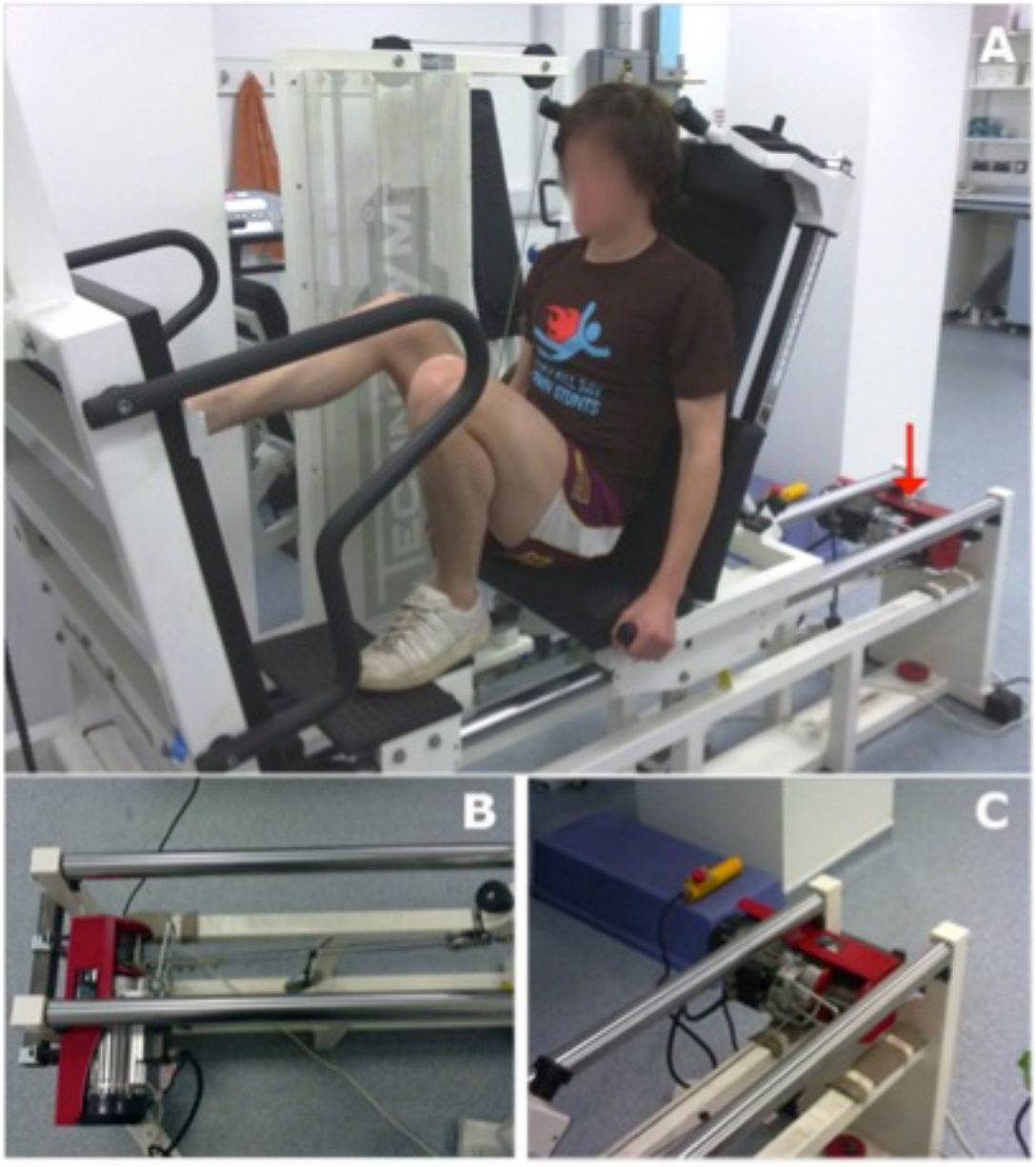


Fig 2. VL ultrasound image captured at rest: pennation angle and the visible part of a muscle fascicle is shown.

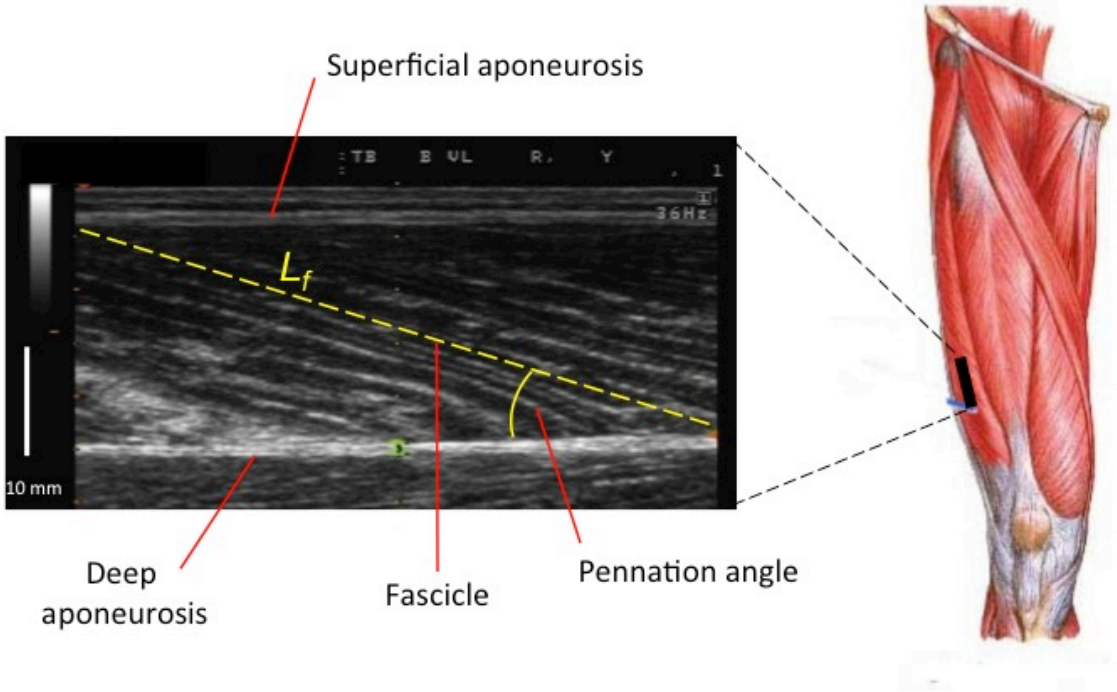


Fig 3. Post/Pre training ratios of muscle volume, isometric MVC and muscle architecture in the concentric and eccentric exercise groups. Y = 1 represent the baseline value. Data normalized to pre values; means ± SEM (** P<0.05 ** P<0.001 *** P<0.0001 - ^^, ^^^ = = significantly different between groups: P < 0.01 and P < 0.001, respectively*).

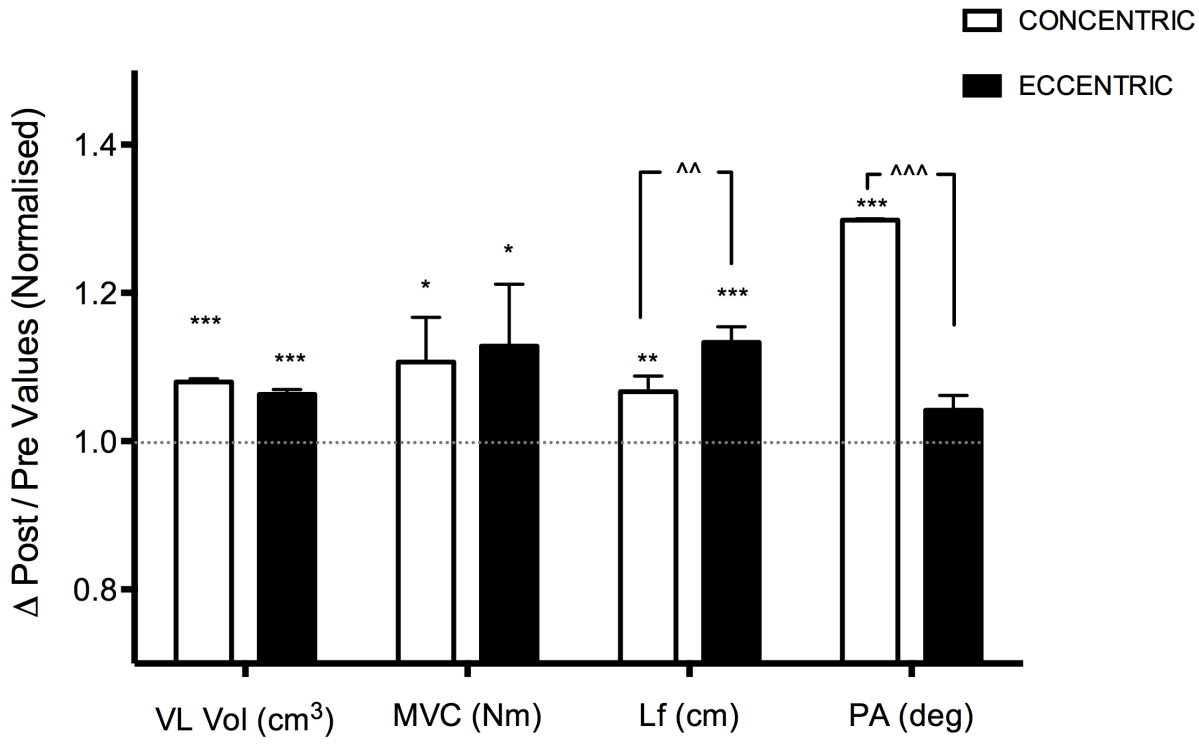


Fig 4. Regional Hypertrophy of VL muscle (ACSA = Anatomical Cross Sectional Area) after concentric and eccentric training. Data are means ± SEM (***P < 0.01*
****P < 0.001 - ^, ^^ = significantly different between groups: P < 0.05, P < 0.01*).

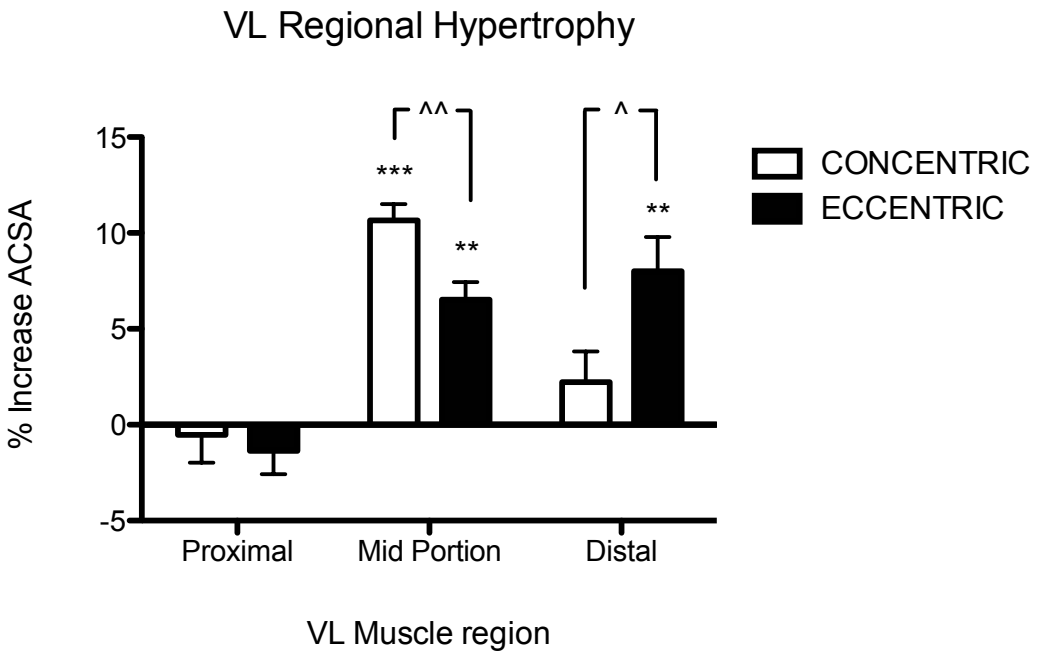


Fig 5. Muscle architectural behavior during a concentric and eccentric contraction performed on the leg-press device (90° to 170° knee joint angle, 180° = anatomical zero). Y = 1 represent the baseline value. Data normalized to pre values; means ± SEM (**** P < 0.0001 - ^^^ = significantly different between groups P < 0.0001*).

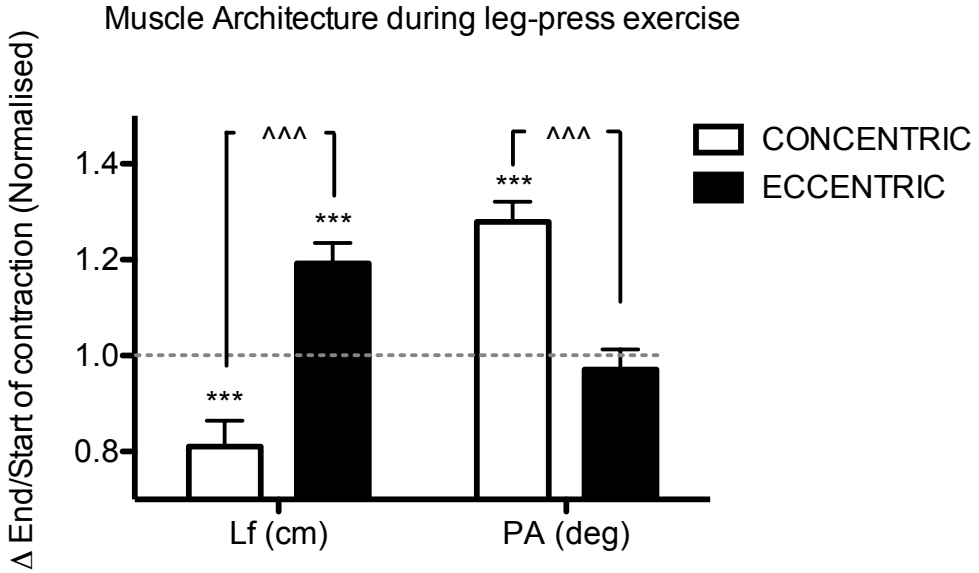
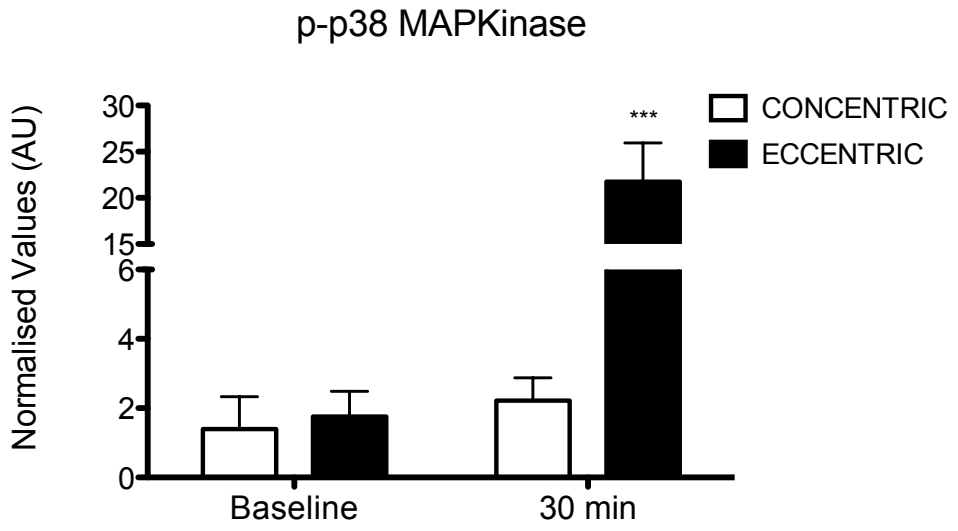
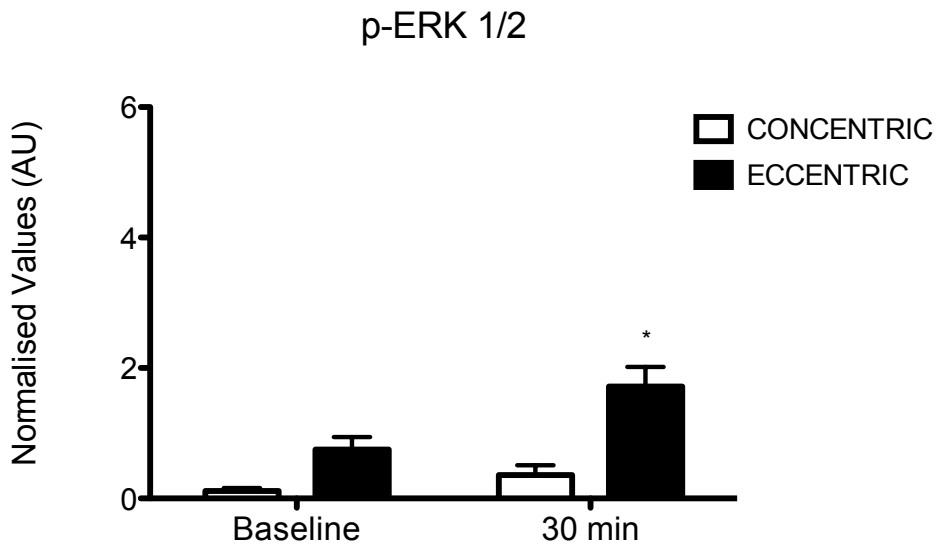


Fig 6. MAPK Molecular responses (phosphorylation) at 30 minutes after either a single concentric or eccentric training session. Data are means ± SEM. (** P<0.05 , **P < 0.01, ***P < 0.001*)


p-p38 MAPKinase
Normalised Values (AU)
30
25
20
15
6
4
2
0
***
CONCENTRIC
ECCENTRIC
Baseline
30 min


p-ERK 1/2
Normalised Values (AU)
6
4
2
0
*
CONCENTRIC
ECCENTRIC
Baseline
30 min

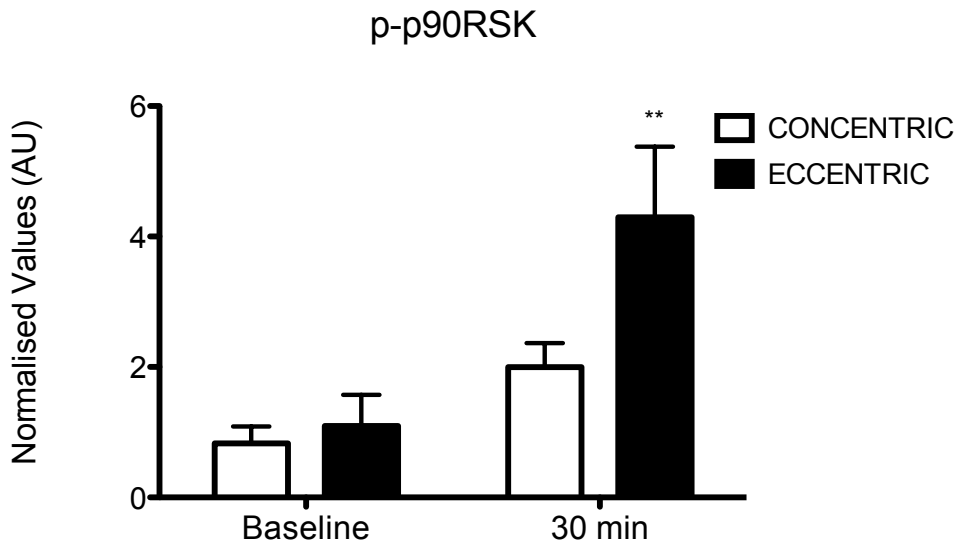
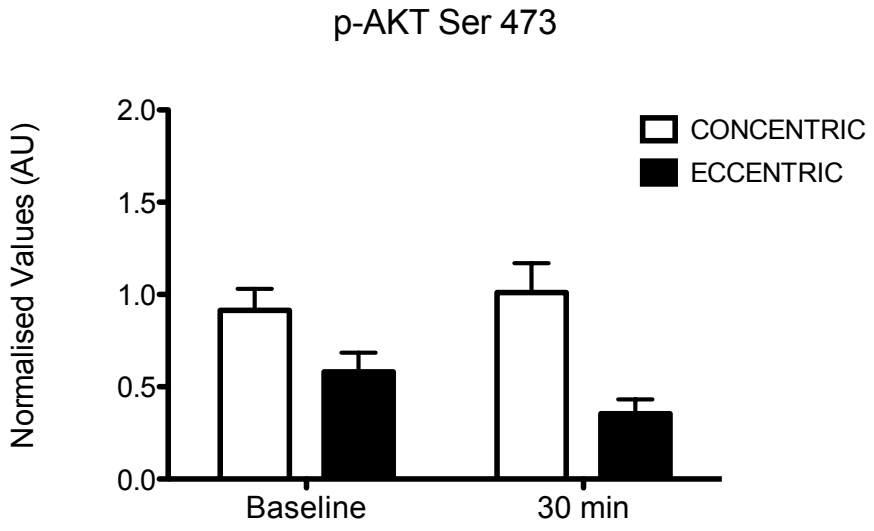
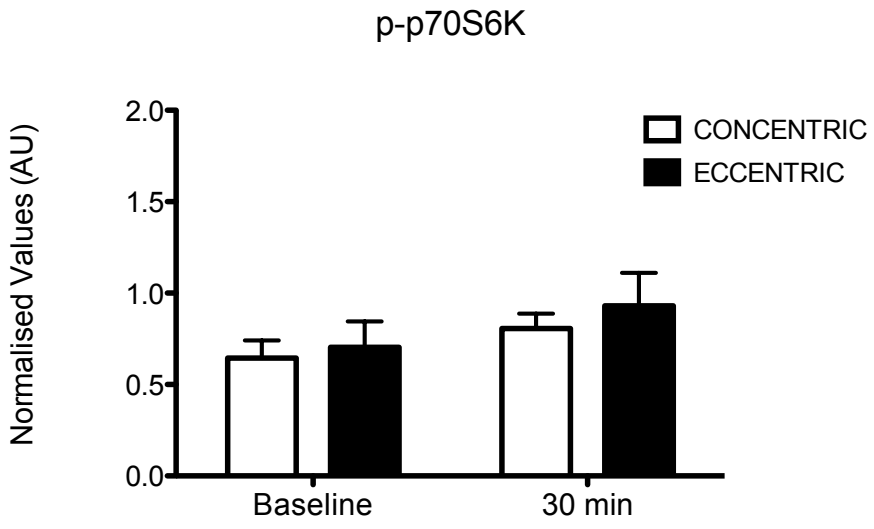

p-p90RSK
Normalised Values (AU)
6
4
2
0
**
CONCENTRIC
ECCENTRIC
Baseline
30 min

Fig 7. Akt, p70S6K and 4EBP1 molecular responses (phosphorylation) at 30 minutes after either a single concentric or eccentric training session. Data are means ± SEM (** P<0.05*).


p-AKT Ser 473
Normalised Values (AU)
2.0
1.5
1.0
0.5
0.0
CONCENTRIC
ECCENTRIC
Baseline
30 min


p-p70S6K
Normalised Values (AU)
2.0
1.5
1.0
0.5
0.0
CONCENTRIC
ECCENTRIC
Baseline
30 min

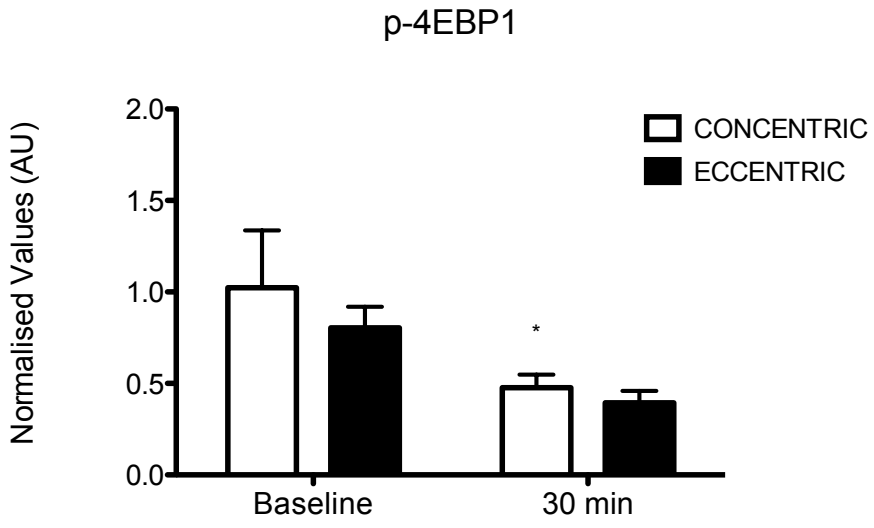

p-4EBP1
Normalised Values (AU)
2.0
1.5
1.0
0.5
0.0
CONCENTRIC
ECCENTRIC
*
Baseline
30 min

Table 1. Maximum lifting or lowering ability changes for the CON Group (CON) and the ECC one (ECC). EMG values were recorded only at baseline during 1RM leg-press for concentric and eccentric phases. Load ratio is also showed and calculated as the ratio of pre and post ECC/CON training loads.

CON 1RM (Kg)			**ECC 1RM (Kg)**			**Load ratio**	
Pre	**Post**	**Δ%**	**Pre**	**Post**	**Δ%**	**Pre**	**Post**
192 ± 16	262 ± 30	36*	233 ± 13	337 ± 9	44*	1.21	1.29
EMG (mV)			**EMG (mV)**				
0.33± 0.1			0.31± 0.1				

(Pre = baseline, Post = Post-training) values are means ± SEM (** P<0.05, pre-to-post difference*).